



Universiteit
Leiden
The Netherlands

Cytokine-mediated regulation of immunity during persistent viral infection

Pratumchai, I.

Citation

Pratumchai, I. (2022, September 20). *Cytokine-mediated regulation of immunity during persistent viral infection*. Retrieved from <https://hdl.handle.net/1887/3459110>

Version: Publisher's Version

License: [Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden](#)

Downloaded from: <https://hdl.handle.net/1887/3459110>

Note: To cite this publication please use the final published version (if applicable).

Chapter 1

General Introduction

Persistent viral infection and Lymphocytic Murine Choriomeningitis Virus as a model system

Chronic viral infections such as human immunodeficiency virus (HIV), Hepatitis C virus (HCV) and hepatitis B virus (HBV) are a major global health issue with hundreds of millions of people infected worldwide(1). HIV infection, which leads to acquired immunodeficiency syndrome (AIDS), has caused more than 35 million deaths globally(2). Although potent antiretroviral therapy has successfully saved many lives in the past decade, the emergence of HIV drug resistance remains a public health concern(3). HBV and HCV can cause serious liver infections that put infected people at high risk of liver cirrhosis and cancer(4, 5). Despite tremendous disease burden caused, effective vaccine against HCV is currently unavailable(4). Chronic viral infections are characterized by prolonged viremia, sustained immune activation, disorganized splenic microarchitecture, upregulation of suppressive immunoregulators, and dysfunctional virus-specific T and B cells(6). Since viruses employ different mechanisms to manipulate and escape host immune response, sophisticated cellular interactions within the immunological network are critical to purge the infection. Better understanding of how the immune system respond to persistent viral infections will allow us to determine the pathological consequences of infections and identify effective immunotherapeutic approaches to treat the infections as well as minimize the spread and emergence of drug-resistant virus.

Animal models of persistent viral infection, such as Lymphocytic choriomeningitis virus (LCMV) Clone 13 (Cl-13), has become a critical tool for uncovering fundamental concepts that revolutionize and shape the field of immunology(7). LCMV is an Old World Arenavirus that infects rodents and can be transmitted to humans via saliva, droppings and urine of infected animals(8-11). LCMV genome consists of short (S, approximately 3.5kb) and long (L, approximately 7.2kb) single-stranded RNA segments(12). Infection with LCMV can lead to lymphocytosis and meningeal inflammation especially in immunocompromised individuals and can lead to congenital hydrocephalus and mental retardation in fetuses if infected during pregnancy(13-16). One of the great advantages of LCMV for studying immune responses and determining the cytotoxicity of immune cells is the noncytolytic property, meaning clinical disease and tissue injuries during infection are exclusively caused by the host immune reaction. In addition, different strains and routes of LCMV infection result in wide spectrum of immune responses, allowing the model to be widely used to accurately study pathogenic outcomes of the infection(7). Since the discovery of LCMV in 1934 (17), it has proven to be an invaluable experimental model that contributed to many advancements of our understanding of cellular and humoral immunity. Notably, the use of LCMV has led to the award of the 1996 Nobel Prize in Physiology and Medicine to Peter Doherty and Rolf Zinkernagel for the seminal discovery of major histocompatibility complex (MHC)-restriction of T cell recognition made between 1973-1975 (18, 19). Prior to this discovery, immunological compatibility was a concept studied in transplantation where T lymphocytes could kill foreign cells after recognizing strain-specific membrane proteins differentially expressed on cells of different mouse strains known as MHC antigens. During their studies using LCMV, Zinkernagel and Doherty revealed that T cells must simultaneously recognize both foreign and self MHC antigens to kill infected cells. The key experiment was performed by mixing LCMV specific cytotoxic T lymphocytes (CTL) derived from mice with different (histocompatibility-2) H-2 haplotypes with target cells which were LCMV-infected mouse fibroblasts (L929) derived from CBA/H mice with an H-2^{k/k} allele. They examined the ability of CTL to kill target cells and observed that CTL derived from mice with at least one allele that is H-2k could specifically killed infected L929 cells(20). This finding further

allowed us to understand that once foreign peptide is processed, MHC molecules bind to peptide fragments and present them to T cells in a form of peptide-MHC complex (pMHC). T-cells receptors (TCRs) then recognize pMHC and mediate killings of infected cells. This paradigm-shifting discovery has advanced our knowledge of how antigen-specific T cells recognize virus infected cells and enabled us to develop more tools such as tetramers and TCR transgenic mice that made LCMV an invaluable system for studying cellular immune response during persistent viral infection(21). Remarkably, MHC tetramers have been widely used to accurately detect and measure functions of antigen-specific T cells. The design and utilization of tetramer was first described in 1996 in the laboratory of Mark Davis(22). To generate MHC tetramers, a known epitope is loaded on an MHC molecule of the allele that most optimally presents the epitopes to create pMHC. Then, the four loaded soluble biotinylated pMHC were mixed with fluorochrome-conjugated streptavidin to create tetramerization of MHC monomers that can stably bind to TCRs. After staining with MHC tetramer, antigen-specific T cells can then be evaluated using standard flow cytometry.

Given that virus-specific T cells are essential for antiviral immunity, TCR transgenic (Tg) mice with high frequencies of certain TCRs were developed(23). This allows a large number of antigen-specific T cells to be obtained and used to gain in-depth understanding of how antigen-specific T cells regulate antiviral immunity. The two commonly used TCR Tg mice to study T cell responses during LCMV infections are P14 and SMARTA TCR Tg mice. P14 TCR Tg mice have CD8 T cells repertoire that expresses a rearranged TCR (Tcra-V2, Tcra-J TA31 / Tcrb-V8.1, Tcrb-D, Tcrb-J 2.4) specifically recognizing MHC-I-specific LCMV glycoprotein (GP) 33–41 peptide and do not develop endogenous mature TCR alpha beta cells(23). P14 TCR Tg mice enabled scientists to study CD8 T effector functions and memory formation during acute LCMV infection as well as T cell exhaustion and viral pathogenesis during persistent LCMV infection(24). Similar to P14 mice, SMARTA TCR Tg mice do not develop endogenous mature TCR alpha beta cells but have CD4 T cells repertoire that expresses a rearranged TCR (V α 2.3-J α DK1 and V β 8.3-J β 2.5) specifically recognizing LCMV glycoprotein (GP) 61–80 H2-Ab complexes(25). SMARTA TCR Tg mice are used to study the role of virus specific CD4 T cells in supporting CD8 T cells functions and promoting viral clearance.

The original clone of LCMV, which is LCMV Armstrong (LCMV-Arm), was isolated in 1934 by Charles Armstrong, whom the virus was named after(17). In 1984, several mutant strains of LCMV-Armstrong were isolated by Rafi Ahmed, a postdoctoral fellow in the Oldstone laboratory(26). One of the clones, Arm53b, was isolated and observed to causes acute infection with severe inflammation in the meninges that can be resolved within 10 days. On the contrary, clone number 13 (LCMV Cl-13) can establish persistent infection that last up to 3 months despite having only 3 amino acids different from those of LCMV-Arm(26). The ability of LCMV Cl-13 to establish persistent infections is governed by two amino acids, one phenylalanine to leucine mutation at position 260 of the viral glycoprotein (GP260) and another lysine to glutamine mutation within the polymerase (L1079)(27). The third asparagine to aspartic acid mutation of GP176 is not critical for viral persistence(27, 28). Mechanistically, mutation of GP260 contributes to viral persistence by increasing receptor binding affinity causing LCMV Cl-13 to infect larger number and wider range of cells including DCs and macrophages. Infected DCs exhibit reduced expression of MHC I, MHC II, CD40 and CD80c causing DCs to be less efficient at presenting antigens and inducing T cell activation. Unlike GP260 mutation, mutation of L1079 contributes to viral persistence by increasing viral load and prolonging the infection(27-32), causing T cell exhaustion.

T cell exhaustion is a phenomenon reported in chronic viral infections as well as in cancer where antigen-specific CD8 T cells lose their effector functions in a hierarchical manner(6, 33-35). At early stage of T cell exhaustion, the loss of interleukin (IL)-2 is apparent before a subsequent loss of Tumor Necrosis Factor alpha (TNF α) is observed at the intermediate stage. As antigen loads remain high, T cells enter the severe stage of exhaustion where antigen-specific T cells lose their ability to produce interferon- γ (IFN- γ) as well as beta-chemokines(36-38). In addition, exhausted T cells display reduced cytolytic functions. Together, these events lead to the final stage of exhaustion where exhausted T cells ultimately undergo apoptosis and physical deletion causing dramatic reduction in the number of virus-specific T cells during viral persistence(35, 38). Another key characteristic of T cell exhaustion is increased expression of negative immunoregulator such as IL-10 and inhibitory receptors (IRs) such as programmed cell death protein 1 (PD-1), cytotoxic T-Lymphocyte Associated Protein 4 (CTLA-4), T cell immunoglobulin and mucin domain 3 (Tim-3), Lymphocyte-activation gene (LAG-3), CD160, and 2B4(39-45). IRs are checkpoint proteins that regulate activating receptors in T cells. Increased expression of IRs constrains T cell functions and causes T cell exhaustion. Immune checkpoint blockades using monoclonal antibodies against CTLA-4 and PD-1 have been proven successful not only in restoring T cell functions but also in purging chronic viral infection as well as controlling cancers(39, 46). The success of immunotherapies targeting PD-1 and CTLA-4 led to the award of the 2018 Nobel Prize in Physiology and Medicine to James Allison for the discovery of CTLA-4 and Tasuko Honjo for the discovery of PD-1(46-52). After the discovery of CTLA-4 in 1978, Allison revealed the inhibitory effect it has on T cell functions and developed blocking antibody against CTLA-4. In a mouse model of colon carcinoma, blocking CTLA-4 increased tumor rejection, suggesting that immune checkpoint inhibition can be used as a therapeutic strategy for cancer treatment. Moreover, a significant advancement in immunotherapy was made when the role of PD-1 in promoting T cell exhaustion was described using LCMV Cl-13 by Daniel Barber, a postdoctoral fellow in the laboratory of Rafi Ahmed(39). Barber showed that exhausted T cells upregulate PD-1 during persistent LCMV infection. By blocking PD-1/PD-L1, the group were able to reverse T cell exhaustion and significantly accelerate viral clearance. Following these observations, multiple groups reported that PD-1 expression is associated with T cell exhaustion in HIV infected patients(53-56). Subsequent study demonstrated that blocking PD-1 during SIV infected non-human primates improved virus-specific CD8 T cell response, promoted memory B cell proliferation, enhanced SIV-specific antibody production, decreased viral load, and improved survival of infected animals(57, 58). Similar observation has been made in HCV infected non-human primates(59-61), suggesting that PD-1 blockade could potentially be an immunotherapeutic strategy for controlling T cell exhaustion in human viral infections and possibly cancers. Although the use of PD-1 checkpoint blockade is currently being assessed in human chronic viral infection, the success in using anti-PD-1 immunotherapy in treating cancers has been clinically proven(62). Taken together, these observations demonstrate that LCMV Cl-13 has been a powerful tool that enables us to understand the concept of MHC restriction, T cell exhaustion and immunotherapy. Despite the success of immune checkpoint inhibitors (ICIs) in revolutionizing the practice of medical oncology, treatment failure, resistance and relapse are frequently observed(63, 64). More studies are hence needed to improve our ability to better predict potential responders and develop new combination therapies for those who do not respond to standard ICI treatments. Recent findings identified a subset of stem-like virus-specific TCF1⁺ Tim3⁻ CD8 T cells that gives rise to the proliferative burst during anti-PD1/PD-L1 immunotherapy(41, 65). New immunotherapeutic

approaches targeting this subset of CD8 T cells combines with traditional ICI treatments could potentially increase the number of patients responding to ICIs.

Cytokine signaling in viral infection and their potential for clinical therapy

Host-derived immune regulators such as cytokines are important immunomodulators that have crucial roles during persistent viral infection. Cytokines are soluble proteins that mediate cell-to-cell communication and interaction to facilitate critical stimulatory or suppressive effects, which in turn result in preventing or promoting viral persistence. Significant research efforts from both humans and mice have identified multiple cytokines that play critical roles during persistent viral infections(66, 67).

IL-10 signaling

Interleukin-10 (IL-10) is an immunosuppressive cytokine that contributes to viral persistence(68, 69). It is produced by several immune cells including dendritic cells (DCs), monocytes, macrophages, B cells, CD4 T cells, CD8 T cells and T regulatory T cells shortly after infection(70, 71). IL-10 signals through a heterodimeric type II cytokine receptor composed of IL-10R1 and IL-10R2 subunits to activate the janus tyrosine kinases JAK1 and Tyk2 and the signal transducers and activators of transcription STAT1, STAT3, and STAT5 (72-75). Elevated IL-10 level was observed in patients with chronic viral infections including EBV, HBV, HCV, and HIV (76-80). Blockade of IL-10 resulted in increased proliferation and cytokine secretion of T cells isolated from HIV And HCVs. Similar phenomenon was observed in mice infected with persistent LCMV Cl-13 (68, 81). Faster clearance of persistent LCMV infection was observed in IL-10 deficient mice as well as in mice treated with anti-IL-10R-specific antibodies at early-stage post-infection (p.i.)(68). These mice displayed improved functions of CD4 and CD8 T cells as well as faster clearance. Mechanistically, IL-10 exerts its immunosuppressive effects on T cells by altering DC maturation via reducing the expression of the co-stimulatory molecules CD80 and MHC class I and II(69, 75). Consequently, IL-10-primed DCs exhibit reduced ability to prime CD4 Th1 cells causing CD4 T cells to become suppressive IL-10 producing cells instead of being IFN γ -producing Th1 cells. A recent study demonstrated that co-treatments of anti-IL-10R and anti-PD-L1 antibodies further enhanced T cell functions and accelerated viral clearance in mice infected with persistent LCMV Cl-13 (82). Given that IL-10 plays important roles during persistent viral infection, further understanding of the IL-10 signaling network will improve our fundamental knowledge of persistent viral infection and potentially lead to therapeutic strategies that can be used to treat chronic viral infections in human.

IL-2 signaling

As host immune system fails to control the infection and antigen levels subsequently increase, CD8 T cells start to express inhibitory receptors such as PD-1, Lag-3, and Tim-3 before gradually losing their effector functions in a process known as T cell exhaustion. Several cytokines have been shown to positively regulate antiviral CD8 T cells and promote viral clearance(67). IL-2 is a member of a γ_c -dependent cytokine family. It was identified as a powerful growth factor for T cells and was the first cytokine to be molecularly cloned after its discovery in 1983(83, 84). During viral infection, IL-2 is primarily produced by CD8 and CD4 T cells and binds to a receptor complex composed of IL-2R α (CD25), IL-2 β (CD122), and γ_c (CD132) subunits(85-87). Significant

findings using a mouse model of persistent LCMV infection showed that IL-2 signaling is essential for the maintenance of virus-specific CD8 T cells and administration of IL-2 improved CD8 T cell response while promoting faster control of the virus(88). Intriguingly, a combination of IL-2 administration with PD-L1 blockade substantially enhanced expansion of antiviral CD8 T cells and reduced viral load(89), suggesting that IL-2 could potentially be used as treatment for persistent viral infections in human. However, several clinical trials in HIV patients revealed that IL-2 administration was able to increase numbers of CD4 but unable to reduce viral load(90). Together, these findings suggest that even though IL-2 is a promising immunotherapeutic cytokine to treat viral persistence, better understanding of the biology of IL-2 is required to achieve the optimal therapeutic outcome.

IL-7 signaling

Another cytokine from a γ_c -dependent cytokine family that positively regulates CD8 T cells during chronic viral infection is IL-7. Previous studies have shown that IL-7 plays a key role during development, maintenance, and survival of T cells(91-94). IL-7 is largely produced by stromal cells and signals through a dimeric receptor complex consisting of IL-7 α (CD127) and γ_c (CD132) subunits to activate JAK1, JAK3 and STAT5(95). Recent studies demonstrated that administration of IL-7 during chronic LCMV infection promotes expansion of virus-specific CD8 T cells as well as increased numbers of CD4, CD8 and B cells causing faster clearance of the virus(96, 97). The unique capacity of IL-7 to improve host immune response and promote clearance of persistent viral infection was further explored during HIV and simian immunodeficiency virus (SIV) infections. Concomitantly, IL-7 administration in patients infected with HIV and primates infected with SIV led to increase of circulating CD4 and CD8 T cells(98, 99). Taken together, these findings uncovered that IL-7 is another cytokine with promising therapeutic effects that could potentially be used to treat persistent viral infection.

IL-21 signaling

Besides IL-2 and IL-7, studies have recently identified IL-21, another member of the γ_c -dependent cytokine family, as a powerful positive immunoregulator. IL-21 is mainly produced by different subsets of CD4 T cells and signals through a heterodimeric receptor complex consisting of the γ_c (CD132) and an IL-21-receptor (IL21R) (CD360) subunits(100-102). It signals on CD4 T cells, CD8 T cells, B cells and natural killer (NK) cells to activate JAK1, JAK3, STAT1, STAT3 and STAT5(103). Remarkable findings by three different research groups using a mouse model of persistent LCMV infection showed that IL-21R signaling is required for the maintenance of effective antiviral CD8 T cells(104-106). Mice deficient in IL-21R displayed severely impaired functions of CD8 T cells during persistent LCMV infection. Meanwhile exogenous treatment of IL-21 in chronically infected mice led to increased number and improved functions of antiviral CD8 T cells as well as reduced viral titers(106). Given the potential of IL-21 as a promising therapeutic agent for chronic viral infection, further work is required to determine the effects of IL-21 immunotherapy in different conditions and disease models to gain deeper insights on the mechanisms by which IL-21 supports effector functions of CD8 T cells and promote viral clearance. Moreover, understanding of where this vital cytokine is produced and how IL-21-producing cells are maintained will enable us to determine better therapeutic strategies to treat persistent viral infections.

IL-6

IL-6 is an important cytokine that supports differentiation and maintenance of Tfh cells by inducing expression of genes associated with Tfh differentiation such as Bcl-6, CXCR5 and IL-21(107, 108). IL-6 is produced mainly by follicular dendritic cells (FDC) during late stage of chronic viral infection and can also be produced by other cell types such as T cells, B cells, mast cells, macrophages, and dendritic cells in different disease models(109). IL-6 signals through a receptor complex composed of IL-6R α and gp130, which is shared with IL-27, to activate primarily STAT3. Observations of patients infected with HIV, HBV and HCV infections revealed that reduced levels of IL-6 were associated with disease progression, suggesting IL-6 plays an important role in promoting antiviral immunity(110-112). IL-6 promotes antiviral immunity by supporting Tfh differentiation and promoting accumulation of GCB as well as production of virus-specific antibody. Consequently, IL-6 signaling during persistent viral infection leads to improved CD8 T cell functions, increased neutralizing antibody production, and faster viral control. Further study demonstrated that T cell-specific gp130 signaling is required for IL-21 production by CD4 T cells and clearance of LCMV Cl-13 infection(113). Given that IL-27 also signals through a receptor complex consisting of gp130, the authors further explored the role of IL-27 during persistent LCMV infect and uncovered that IL-27 is also required for control of LCMV Cl-13 infection and maintenance of virus-specific CD4 T cells(113).

IL-27 signaling

IL-27 is a heterodimeric cytokine in the IL-6 and IL-12 family composed of the IL-27p28 and Epstein Barr-Virus Inducible protein-3 (EBI3) subunits(114). It signals through a heterodimeric receptor consisting of WSX-1 and gp130, which is shared with IL-6 receptor complex to convey IL-27 signal through activating of STAT1 and STAT3 (115). IL-27 is produced and secreted by dendritic cells and macrophages upon stimulation with pathogen derived inflammatory signals through Toll-Like receptors (TLRs)(116-118). Expression of IL-27 receptor has been described in multiple cell types including naïve T cells, macrophages, B cells, endothelial cells, monocytes and activated dendritic cells, thus this cytokine has the potential to regulate multiple arms of the immune response. In addition, an interesting recent observation revealed that IL-27 is likely expressed by CX3CR1⁺ sympathetic-neuron-associated macrophages (SAMs) to promote control of obesity(119). Initial investigation of IL-27 receptor deficient mice revealed an important role for IL-27 signaling for induction of T helper type I (Th1) responses(120). Subsequent studies demonstrated a crucial role for IL-27 in eliminating intracellular pathogens(121, 122). Moreover, several reports have demonstrated a pathogenic role for IL-27 signaling in arthritis models in rats and experimental autoimmune encephalomyelitis (EAE) in mice(123-125). While the above findings demonstrate a clear role for IL-27 in promoting inflammatory responses, several studies have reported robust anti-inflammatory functions attributable to IL-27 signaling(126). The absence of IL-27 receptor leads to immune pathology due to elevated pro-inflammatory cytokines from T cells during protozoan infections(127). Similar to protozoan infection, mycobacterium tuberculosis infection of IL-27R KO mice lead to increased lung and liver immune pathology(128). Further, IL-27 signaling can limit the production of IL-2 during Th1 responses however the mechanism of IL-2 regulation is still a matter of debate(129-131). Moreover IL-27 signaling has been shown to suppress Th17 differentiation and induce elevated levels of the immune suppressive cytokine, IL-10(123, 132, 133). Thus, IL-27 signaling has been associated with both pro- and anti-inflammatory functions unique to the specific pathogen being studied. However, how IL-27 signaling regulates the CD8 T cell response to viral infection and cancer remains equally

interesting and complex(134). Following acute Corona and Influenza viral infections, IL-27 attenuates anti-viral CD8 T cell responses(135, 136). However, during chronic antigen persistence and inflammation, IL-27 can also have pleiotropic effects; Following chronic infection with LCMV clone-13, IL-27 deficient mice display severe T cell dysfunction and are unable to control the infection(113). IL-27 signaling is essential for promoting survival of virus-specific CD4 T cells at later stage of infection. Reduction in frequency and total number of virus-specific CD4 T cells were observed in addition to reduced LCMV-specific IgG2a/2C in IL-27ra KO mice(113). In contrast, others have reported that IL-27 signaling promotes the expression of negative immune regulatory molecules (TIM3, PD-1 etc.) on CD8 T cells in both C113 infection and cancer and can be detrimental to cancer immunity(137, 138). In the context of anti-tumor responses, IL-27 has been reported to both promote (139-141)and restrain(137, 138) T cell-mediated immune control by various mechanisms. In addition, during Ovalbumin priming IL-27 also promotes IL-21 production(142), which is required for promoting CD8 T cell function, antibody production and viral clearance(104-106). Disruption of IL-27 signaling causes reduction in numbers of virus-specific CD4 T cells and LCMV-specific antibody at later stage of infection(113). Overall, the regulation of CD8 and CD4 T cell responses by IL-27 is complex, and gaps in our knowledge remain about the cellular sources of IL-27 and mechanistically how this cytokine simultaneously drives immune stimulatory and suppressive properties in T cells.

Interferon signaling

IFN-I were initially known for their antiviral properties during acute viral infection. Paradoxically, sustained IFN-I signaling during persistent viral infections were reported by multiples studies to cause deleterious effects to host immune response leading to immune suppression and viral persistence(143). IFN-I are a large family of cytokines consisting of the well characterized subtypes IFN α and IFN β as well as the lesser defined subtypes IFN ω , IFN ϵ , IFN δ , IFN τ and IFN ζ . After viral sensing via pathogen recognition receptors (PPRs) recognizing pathogen-associated molecular patterns (PAMPS), IFN-I production is induced with IFN α being produced largely by plasmacytoid dendritic cells (pDCs)(144). Upon secretion, IFN-I signal through a heterodimeric transmembrane receptor complex composed of Interferon Alpha And Beta Receptor Subunit 1 (IFNAR1) and Interferon Alpha And Beta Receptor Subunit 2 (IFNAR2) subunits to phosphorylate JAK1 and Tyk2 in order to activate STAT1 and STAT2(145). Upon activation, STAT1 and STAT2 can assemble with Interferon Regulatory Factor 9 (IRF9) to form a trimeric transcription factor, IFN-stimulated gene factor 3, to trigger the expression of interferon-stimulated genes (ISGs), which encodes antiviral proteins(146, 147). Despite having antiviral functions, prolonged IFN-I signaling and elevated level of ISGs during HIV and SIV infections were found to be associated with disease progression and reduced number of CD4 T cells(148-150). Similarly, persistent LCMV infection in mice resulted in prolonged IFN-I signaling that contributes to viral persistence by causing disorganized lymphoid secondary lymphoid organs, reduced MHC I and II expression in DC, as well as increased PD-L1 and IL-10 expression(151, 152). Blocking IFN-I receptor at the beginning of infection by using antibody against IFNAR1 accelerated viral clearance and improved CD4 T cell response in addition to reducing IL-10 and PD-L1 expression. Furthermore, blockade of IFN β also improved functions of CD4 T cells and facilitated faster control of the infection when blockade of IFN α failed to exert the same effects(153). These findings suggest that IFN β plays a dominant role in causing immune suppression during persistent viral infection when compared with IFN α . Recent studies revealed that IFN-I signaling during chronic viral infection suppresses B cells and inhibits the formation of the stem-like virus-specific

TCF1⁺CD8 T cells that gives rise to proliferative burst after PD-1 blockade(154-157). Since IFNAR blockade resulted in increased TCF1⁺ CD8 T cells, it would be interesting to see whether blocking IFN-I can augment the ability of anti-PD-1 immunotherapy to drive CD8 T cell proliferation and viral clearance. Overall, these observations suggest that IFN-I at early stage of infection can be deleterious to the host immune system during persistent viral infection. However, therapeutic strategies targeting IFN-I should be fine-tuned to achieve the most effective outcome with the least possible side-effects given that IFN-I also has other antiviral benefits depending on the timing and type of viral infection. In-depth understanding of these cytokines will allow us to design better immunotherapies through the usage of cytokines, blocking antibodies, or even Jak inhibitors to fight against human disease.

Thesis Outline

This PhD thesis focuses on understanding the biology of IFN-I and IL-27 during persistent LCMV infection. Both cytokines were shown to play crucial but opposing roles in influencing functions, expansion, and survival of virus-specific T cells(113, 151, 152). **Chapter 2** demonstrates the interplay between these two cytokines and the impact that it has on promoting expansion and survival of the stem-like TCF1⁺CD8 T cells that were recently identified as a subset of exhausted CD8 T cells with high proliferative potential after anti-PD-1 treatment. Based on a recent discovery that blockade of type 1 IFN resulted in expansion of the stem-like TCF1⁺ CD8 T cells(154), we demonstrate that this process happens in an IL-27- and STAT1-dependent manner. In addition, we show that IL-27 promotes expression of transcription factor Interferon Regulatory Factor 1 (IRF1) to support proliferation and promote survival of the cells. **In chapter 3**, we discover that B cells secrete IL-27 upon LCMV CI-13 infection and that B-cell-derived IL-27 is required for the control of persistent LCMV infection. We further show that B-cell-derived IL-27 is crucial for accumulations of virus-specific T cells and Tfh cells. B-cell-derived IL-27 also induces IL-21 production by Tfh cells, which subsequently induces antibody class switch. **In chapter 4**, we explore the possibility of using Jak inhibitor ruxolitinib to rescue T cell exhaustion. We demonstrate that ruxolitinib treatment during persistent viral infection is able to increase the total number of the stem-like TCF1⁺ CD8 T cells. In addition, ruxolitinib works in synergy with anti-PD-L1 to promote viral clearance. Further, we examine the impact of ruxolitinib in preclinical tumor model as well as in a clinical trial of Hodgkin lymphoma resistant to checkpoint inhibitors. We show that ruxolitinib positively regulates T cells and enhanced checkpoint blockade.

References

1. H. W. Virgin, E. J. Wherry, R. Ahmed, Redefining chronic viral infection. *Cell* **138**, 30-50 (2009).
2. C. Del Rio, The global HIV epidemic: What the pathologist needs to know. *Semin Diagn Pathol* **34**, 314-317 (2017).
3. R. K. Gupta *et al.*, HIV-1 drug resistance before initiation or re-initiation of first-line antiretroviral therapy in low-income and middle-income countries: a systematic review and meta-regression analysis. *Lancet Infect Dis* **18**, 346-355 (2018).
4. M. P. Manns *et al.*, Hepatitis C virus infection. *Nature Reviews Disease Primers* **3**, 17006 (2017).

5. L. S. Y. Tang, E. Covert, E. Wilson, S. Kottlilil, Chronic Hepatitis B Infection: A Review. *Jama* **319**, 1802-1813 (2018).
6. E. J. Wherry, T cell exhaustion. *Nat Immunol* **12**, 492-499 (2011).
7. M. B. Oldstone, An Odyssey to Viral Pathogenesis. *Annu Rev Pathol* **11**, 1-19 (2016).
8. R. M. Roebroek, B. H. Postma, U. J. Dijkstra, Aseptic meningitis caused by the lymphocytic choriomeningitis virus. *Clin Neurol Neurosurg* **96**, 178-180 (1994).
9. R. Deibel, G. D. Schryver, Viral antibody in the cerebrospinal fluid of patients with acute central nervous system infections. *J Clin Microbiol* **3**, 397-401 (1976).
10. R. L. Warkel *et al.*, Fatal acute meningoencephalitis due to lymphocytic choriomeningitis virus. *Neurology* **23**, 198-203 (1973).
11. L. L. Barton, M. B. Mets, Congenital lymphocytic choriomeningitis virus infection: decade of rediscovery. *Clin Infect Dis* **33**, 370-374 (2001).
12. Y. Riviere, P. J. Southern, R. Ahmed, M. B. Oldstone, Biology of cloned cytotoxic T lymphocytes specific for lymphocytic choriomeningitis virus. V. Recognition is restricted to gene products encoded by the viral S RNA segment. *J Immunol* **136**, 304-307 (1986).
13. R. T. Bechtel, K. A. Haught, M. B. Mets, Lymphocytic choriomeningitis virus: a new addition to the TORCH evaluation. *Arch Ophthalmol* **115**, 680-681 (1997).
14. R. Wright *et al.*, Congenital lymphocytic choriomeningitis virus syndrome: a disease that mimics congenital toxoplasmosis or Cytomegalovirus infection. *Pediatrics* **100**, E9 (1997).
15. G. Enders, M. Varho-Göbel, J. Löhler, E. Terletskaia-Ladwig, M. Eggers, Congenital lymphocytic choriomeningitis virus infection: an underdiagnosed disease. *Pediatr Infect Dis J* **18**, 652-655 (1999).
16. L. L. Barton, M. B. Mets, C. L. Beauchamp, Lymphocytic choriomeningitis virus: emerging fetal teratogen. *Am J Obstet Gynecol* **187**, 1715-1716 (2002).
17. C. Armstrong, R. Lillie, Experimental lymphocytic choriomeningitis of monkeys and mice produced by a virus encountered in studies of the 1933 St. Louis encephalitis epidemic. *Public Health Reports (1896-1970)*, 1019-1027 (1934).
18. R. M. Zinkernagel, P. C. Doherty, Restriction of in vitro T cell-mediated cytotoxicity in lymphocytic choriomeningitis within a syngeneic or semiallogeneic system. *Nature* **248**, 701-702 (1974).
19. R. M. Zinkernagel, P. C. Doherty, Immunological surveillance against altered self components by sensitised T lymphocytes in lymphocytes choriomeningitis. *Nature* **251**, 547-548 (1974).
20. R. M. Zinkernagel, P. C. Doherty, The discovery of MHC restriction. *Immunol Today* **18**, 14-17 (1997).
21. M. S. Abdel-Hakeem, Viruses Teaching Immunology: Role of LCMV Model and Human Viral Infections in Immunological Discoveries. *Viruses* **11** (2019).
22. J. D. Altman *et al.*, Phenotypic analysis of antigen-specific T lymphocytes. *Science* **274**, 94-96 (1996).
23. Y. B. Cho, I. G. Lee, Y. H. Joo, S. H. Hong, Y. J. Seo, TCR Transgenic Mice: A Valuable Tool for Studying Viral Immunopathogenesis Mechanisms. *Int J Mol Sci* **21** (2020).
24. H. Pircher *et al.*, Viral escape by selection of cytotoxic T cell-resistant virus variants in vivo. *Nature* **346**, 629-633 (1990).

25. A. Oxenius, M. F. Bachmann, R. M. Zinkernagel, H. Hengartner, Virus-specific MHC-class II-restricted TCR-transgenic mice: effects on humoral and cellular immune responses after viral infection. *Eur J Immunol* **28**, 390-400 (1998).
26. R. Ahmed, A. Salmi, L. D. Butler, J. M. Chiller, M. B. Oldstone, Selection of genetic variants of lymphocytic choriomeningitis virus in spleens of persistently infected mice. Role in suppression of cytotoxic T lymphocyte response and viral persistence. *J Exp Med* **160**, 521-540 (1984).
27. A. Bergthaler *et al.*, Viral replicative capacity is the primary determinant of lymphocytic choriomeningitis virus persistence and immunosuppression. *Proc Natl Acad Sci U S A* **107**, 21641-21646 (2010).
28. B. M. Sullivan *et al.*, Point mutation in the glycoprotein of lymphocytic choriomeningitis virus is necessary for receptor binding, dendritic cell infection, and long-term persistence. *Proc Natl Acad Sci U S A* **108**, 2969-2974 (2011).
29. M. Salvato, P. Borrow, E. Shimomaye, M. B. Oldstone, Molecular basis of viral persistence: a single amino acid change in the glycoprotein of lymphocytic choriomeningitis virus is associated with suppression of the antiviral cytotoxic T-lymphocyte response and establishment of persistence. *J Virol* **65**, 1863-1869 (1991).
30. N. Sevilla *et al.*, Immunosuppression and resultant viral persistence by specific viral targeting of dendritic cells. *J Exp Med* **192**, 1249-1260 (2000).
31. R. Ahmed, M. B. Oldstone, Organ-specific selection of viral variants during chronic infection. *J Exp Med* **167**, 1719-1724 (1988).
32. M. Matloubian, T. Somasundaram, S. R. Kolhekar, R. Selvakumar, R. Ahmed, Genetic basis of viral persistence: single amino acid change in the viral glycoprotein affects ability of lymphocytic choriomeningitis virus to persist in adult mice. *J Exp Med* **172**, 1043-1048 (1990).
33. S. M. Kahan, A. J. Zajac, Immune Exhaustion: Past Lessons and New Insights from Lymphocytic Choriomeningitis Virus. *Viruses* **11** (2019).
34. A. Saeidi *et al.*, T-Cell Exhaustion in Chronic Infections: Reversing the State of Exhaustion and Reinvigorating Optimal Protective Immune Responses. *Front Immunol* **9**, 2569 (2018).
35. A. J. Zajac *et al.*, Viral immune evasion due to persistence of activated T cells without effector function. *J Exp Med* **188**, 2205-2213 (1998).
36. M. J. Fuller, A. Khanolkar, A. E. Tebo, A. J. Zajac, Maintenance, loss, and resurgence of T cell responses during acute, protracted, and chronic viral infections. *J Immunol* **172**, 4204-4214 (2004).
37. M. J. Fuller, A. J. Zajac, Ablation of CD8 and CD4 T cell responses by high viral loads. *J Immunol* **170**, 477-486 (2003).
38. E. J. Wherry, J. N. Blattman, K. Murali-Krishna, R. van der Most, R. Ahmed, Viral persistence alters CD8 T-cell immunodominance and tissue distribution and results in distinct stages of functional impairment. *J Virol* **77**, 4911-4927 (2003).
39. D. L. Barber *et al.*, Restoring function in exhausted CD8 T cells during chronic viral infection. *Nature* **439**, 682-687 (2006).
40. S. D. Blackburn *et al.*, Coregulation of CD8+ T cell exhaustion by multiple inhibitory receptors during chronic viral infection. *Nat Immunol* **10**, 29-37 (2009).
41. S. J. Im *et al.*, Defining CD8+ T cells that provide the proliferative burst after PD-1 therapy. *Nature* **537**, 417-421 (2016).

42. H. T. Jin *et al.*, Cooperation of Tim-3 and PD-1 in CD8 T-cell exhaustion during chronic viral infection. *Proc Natl Acad Sci U S A* **107**, 14733-14738 (2010).
43. R. J. Johnston, X. Yu, J. L. Grogan, The checkpoint inhibitor TIGIT limits antitumor and antiviral CD8(+) T cell responses. *Oncoimmunology* **4**, e1036214 (2015).
44. P. M. Odorizzi, E. J. Wherry, Inhibitory receptors on lymphocytes: insights from infections. *J Immunol* **188**, 2957-2965 (2012).
45. K. Richter, P. Agnellini, A. Oxenius, On the role of the inhibitory receptor LAG-3 in acute and chronic LCMV infection. *Int Immunol* **22**, 13-23 (2010).
46. D. R. Leach, M. F. Krummel, J. P. Allison, Enhancement of antitumor immunity by CTLA-4 blockade. *Science* **271**, 1734-1736 (1996).
47. Y. Ishida, Y. Agata, K. Shibahara, T. Honjo, Induced expression of PD-1, a novel member of the immunoglobulin gene superfamily, upon programmed cell death. *Embo j* **11**, 3887-3895 (1992).
48. E. D. Kwon *et al.*, Manipulation of T cell costimulatory and inhibitory signals for immunotherapy of prostate cancer. *Proc Natl Acad Sci U S A* **94**, 8099-8103 (1997).
49. H. Nishimura, M. Nose, H. Hiai, N. Minato, T. Honjo, Development of lupus-like autoimmune diseases by disruption of the PD-1 gene encoding an ITIM motif-carrying immunoreceptor. *Immunity* **11**, 141-151 (1999).
50. G. J. Freeman *et al.*, Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. *J Exp Med* **192**, 1027-1034 (2000).
51. F. S. Hodi *et al.*, Biologic activity of cytotoxic T lymphocyte-associated antigen 4 antibody blockade in previously vaccinated metastatic melanoma and ovarian carcinoma patients. *Proc Natl Acad Sci U S A* **100**, 4712-4717 (2003).
52. M. J. Smyth, M. W. Teng, 2018 Nobel Prize in physiology or medicine. *Clin Transl Immunology* **7**, e1041 (2018).
53. L. Trautmann *et al.*, Upregulation of PD-1 expression on HIV-specific CD8+ T cells leads to reversible immune dysfunction. *Nat Med* **12**, 1198-1202 (2006).
54. C. Petrovas *et al.*, PD-1 is a regulator of virus-specific CD8+ T cell survival in HIV infection. *J Exp Med* **203**, 2281-2292 (2006).
55. B. E. Palmer *et al.*, In vivo blockade of the PD-1 receptor suppresses HIV-1 viral loads and improves CD4+ T cell levels in humanized mice. *J Immunol* **190**, 211-219 (2013).
56. C. L. Day *et al.*, PD-1 expression on HIV-specific T cells is associated with T-cell exhaustion and disease progression. *Nature* **443**, 350-354 (2006).
57. R. Dyavar Shetty *et al.*, PD-1 blockade during chronic SIV infection reduces hyperimmune activation and microbial translocation in rhesus macaques. *J Clin Invest* **122**, 1712-1716 (2012).
58. V. Velu *et al.*, Enhancing SIV-specific immunity in vivo by PD-1 blockade. *Nature* **458**, 206-210 (2009).
59. L. Golden-Mason *et al.*, Upregulation of PD-1 expression on circulating and intrahepatic hepatitis C virus-specific CD8+ T cells associated with reversible immune dysfunction. *J Virol* **81**, 9249-9258 (2007).
60. N. Nakamoto *et al.*, Functional restoration of HCV-specific CD8 T cells by PD-1 blockade is defined by PD-1 expression and compartmentalization. *Gastroenterology* **134**, 1927-1937, 1937.e1921-1922 (2008).

61. S. Urbani *et al.*, PD-1 expression in acute hepatitis C virus (HCV) infection is associated with HCV-specific CD8 exhaustion. *J Virol* **80**, 11398-11403 (2006).
62. J. M. Fritz, M. J. Lenardo, Development of immune checkpoint therapy for cancer. *J Exp Med* **216**, 1244-1254 (2019).
63. A. Kalbasi, A. Ribas, Tumour-intrinsic resistance to immune checkpoint blockade. *Nature Reviews Immunology* **20**, 25-39 (2020).
64. R. W. Jenkins, D. A. Barbie, K. T. Flaherty, Mechanisms of resistance to immune checkpoint inhibitors. *British Journal of Cancer* **118**, 9-16 (2018).
65. R. He *et al.*, Follicular CXCR5-expressing CD8⁺ T cells curtail chronic viral infection. *Nature* **537**, 412-416 (2016).
66. J. C. Beltra, H. Decaluwe, Cytokines and persistent viral infections. *Cytokine* **82**, 4-15 (2016).
67. M. Hashimoto, S. J. Im, K. Araki, R. Ahmed, Cytokine-Mediated Regulation of CD8 T-Cell Responses During Acute and Chronic Viral Infection. *Cold Spring Harb Perspect Biol* **11** (2019).
68. D. G. Brooks *et al.*, Interleukin-10 determines viral clearance or persistence in vivo. *Nat Med* **12**, 1301-1309 (2006).
69. E. B. Wilson, D. G. Brooks, The role of IL-10 in regulating immunity to persistent viral infections. *Curr Top Microbiol Immunol* **350**, 39-65 (2011).
70. C. T. Ng, M. B. Oldstone, Infected CD8 α - dendritic cells are the predominant source of IL-10 during establishment of persistent viral infection. *Proc Natl Acad Sci U S A* **109**, 14116-14121 (2012).
71. K. Richter *et al.*, Macrophage and T cell produced IL-10 promotes viral chronicity. *PLoS Pathog* **9**, e1003735 (2013).
72. D. S. Finbloom, K. D. Winestock, IL-10 induces the tyrosine phosphorylation of tyk2 and Jak1 and the differential assembly of STAT1 alpha and STAT3 complexes in human T cells and monocytes. *J Immunol* **155**, 1079-1090 (1995).
73. J. Wehinger *et al.*, IL-10 induces DNA binding activity of three STAT proteins (Stat1, Stat3, and Stat5) and their distinct combinatorial assembly in the promoters of selected genes. *FEBS Lett* **394**, 365-370 (1996).
74. R. M. Weber-Nordt *et al.*, Stat3 recruitment by two distinct ligand-induced, tyrosine-phosphorylated docking sites in the interleukin-10 receptor intracellular domain. *J Biol Chem* **271**, 27954-27961 (1996).
75. K. W. Moore, R. de Waal Malefyt, R. L. Coffman, A. O'Garra, Interleukin-10 and the interleukin-10 receptor. *Annu Rev Immunol* **19**, 683-765 (2001).
76. C. Graziosi *et al.*, Lack of evidence for the dichotomy of TH1 and TH2 predominance in HIV-infected individuals. *Science* **265**, 248-252 (1994).
77. M. Clerici *et al.*, Type 1 and type 2 cytokines in HIV infection -- a possible role in apoptosis and disease progression. *Ann Med* **29**, 185-188 (1997).
78. R. P. Woitas *et al.*, HCV-specific cytokine induction in monocytes of patients with different outcomes of hepatitis C. *World J Gastroenterol* **8**, 562-566 (2002).
79. S. Swaminathan, Molecular biology of Epstein-Barr virus and Kaposi's sarcoma-associated herpesvirus. *Semin Hematol* **40**, 107-115 (2003).
80. H. Hofer *et al.*, Bi-allelic presence of the interleukin-10 receptor 1 G330R allele is associated with cirrhosis in chronic HCV-1 infection. *Genes Immun* **6**, 242-247 (2005).

81. M. Ejrnaes *et al.*, Resolution of a chronic viral infection after interleukin-10 receptor blockade. *J Exp Med* **203**, 2461-2472 (2006).
82. D. G. Brooks *et al.*, IL-10 and PD-L1 operate through distinct pathways to suppress T-cell activity during persistent viral infection. *Proc Natl Acad Sci U S A* **105**, 20428-20433 (2008).
83. T. Taniguchi *et al.*, Structure and expression of a cloned cDNA for human interleukin-2. *Nature* **302**, 305-310 (1983).
84. T. M. Kundig *et al.*, Immune responses in interleukin-2-deficient mice. *Science* **262**, 1059-1061 (1993).
85. B. H. Nelson, J. D. Lord, P. D. Greenberg, Cytoplasmic domains of the interleukin-2 receptor beta and gamma chains mediate the signal for T-cell proliferation. *Nature* **369**, 333-336 (1994).
86. B. H. Nelson, D. M. Willerford, Biology of the interleukin-2 receptor. *Adv Immunol* **70**, 1-81 (1998).
87. M. Rickert, X. Wang, M. J. Boulanger, N. Goriatcheva, K. C. Garcia, The structure of interleukin-2 complexed with its alpha receptor. *Science* **308**, 1477-1480 (2005).
88. J. N. Blattman *et al.*, Therapeutic use of IL-2 to enhance antiviral T-cell responses in vivo. *Nat Med* **9**, 540-547 (2003).
89. E. E. West *et al.*, PD-L1 blockade synergizes with IL-2 therapy in reinvigorating exhausted T cells. *J Clin Invest* **123**, 2604-2615 (2013).
90. J. A. Kovacs *et al.*, Controlled trial of interleukin-2 infusions in patients infected with the human immunodeficiency virus. *N Engl J Med* **335**, 1350-1356 (1996).
91. J. T. Tan *et al.*, IL-7 is critical for homeostatic proliferation and survival of naive T cells. *Proc Natl Acad Sci U S A* **98**, 8732-8737 (2001).
92. K. S. Schluns, W. C. Kieper, S. C. Jameson, L. Lefrançois, Interleukin-7 mediates the homeostasis of naïve and memory CD8 T cells in vivo. *Nat Immunol* **1**, 426-432 (2000).
93. T. W. Hand, M. Morre, S. M. Kaech, Expression of IL-7 receptor alpha is necessary but not sufficient for the formation of memory CD8 T cells during viral infection. *Proc Natl Acad Sci U S A* **104**, 11730-11735 (2007).
94. L. M. Bradley, L. Haynes, S. L. Swain, IL-7: maintaining T-cell memory and achieving homeostasis. *Trends Immunol* **26**, 172-176 (2005).
95. C. Kittipatarin, A. R. Khaled, Interlinking interleukin-7. *Cytokine* **39**, 75-83 (2007).
96. M. Pellegrini *et al.*, IL-7 engages multiple mechanisms to overcome chronic viral infection and limit organ pathology. *Cell* **144**, 601-613 (2011).
97. S. G. Nanjappa, E. H. Kim, M. Suresh, Immunotherapeutic effects of IL-7 during a chronic viral infection in mice. *Blood* **117**, 5123-5132 (2011).
98. T. J. Fry *et al.*, IL-7 therapy dramatically alters peripheral T-cell homeostasis in normal and SIV-infected nonhuman primates. *Blood* **101**, 2294-2299 (2003).
99. Y. Levy *et al.*, Enhanced T cell recovery in HIV-1-infected adults through IL-7 treatment. *J Clin Invest* **119**, 997-1007 (2009).
100. C. Holm, C. G. Nyvold, S. R. Paludan, A. R. Thomsen, M. Hokland, Interleukin-21 mRNA expression during virus infections. *Cytokine* **33**, 41-45 (2006).
101. T. Korn *et al.*, IL-21 initiates an alternative pathway to induce proinflammatory T(H)17 cells. *Nature* **448**, 484-487 (2007).
102. R. Nurieva *et al.*, Essential autocrine regulation by IL-21 in the generation of inflammatory T cells. *Nature* **448**, 480-483 (2007).

103. R. Spolski, W. J. Leonard, Interleukin-21: basic biology and implications for cancer and autoimmunity. *Annu Rev Immunol* **26**, 57-79 (2008).
104. H. Elsaesser, K. Sauer, D. G. Brooks, IL-21 Is Required to Control Chronic Viral Infection. *Science* **324**, 1569-1572 (2009).
105. A. Fröhlich *et al.*, IL-21R on T Cells Is Critical for Sustained Functionality and Control of Chronic Viral Infection. *Science* **324**, 1576-1580 (2009).
106. J. S. Yi, M. Du, A. J. Zajac, A Vital Role for Interleukin-21 in the Control of a Chronic Viral Infection. *Science* **324**, 1572-1576 (2009).
107. J. A. Harker, G. M. Lewis, L. Mack, E. I. Zuniga, Late interleukin-6 escalates T follicular helper cell responses and controls a chronic viral infection. *Science* **334**, 825-829 (2011).
108. Y. S. Choi, D. Eto, J. A. Yang, C. Lao, S. Crotty, Cutting edge: STAT1 is required for IL-6-mediated Bcl6 induction for early follicular helper cell differentiation. *J Immunol* **190**, 3049-3053 (2013).
109. J. A. Harker, K. A. Wong, A. Dolgoter, E. I. Zuniga, Cell-Intrinsic gp130 Signaling on CD4⁺ T Cells Shapes Long-Lasting Antiviral Immunity. *J Immunol* **195**, 1071-1081 (2015).
110. D. L. Birx *et al.*, Induction of interleukin-6 during human immunodeficiency virus infection. *Blood* **76**, 2303-2310 (1990).
111. D. Torre *et al.*, Serum levels of interleukin-1 alpha, interleukin-1 beta, interleukin-6, and tumor necrosis factor in patients with acute viral hepatitis. *Clin Infect Dis* **18**, 194-198 (1994).
112. N. E. Spanakis *et al.*, Cytokine serum levels in patients with chronic HCV infection. *J Clin Lab Anal* **16**, 40-46 (2002).
113. J. A. Harker, A. Dolgoter, E. I. Zuniga, Cell-intrinsic IL-27 and gp130 cytokine receptor signaling regulates virus-specific CD4⁺ T cell responses and viral control during chronic infection. *Immunity* **39**, 548-559 (2013).
114. R. A. Kastelein, C. A. Hunter, D. J. Cua, Discovery and biology of IL-23 and IL-27: related but functionally distinct regulators of inflammation. *Annu Rev Immunol* **25**, 221-242 (2007).
115. K. Hirahara *et al.*, Asymmetric Action of STAT Transcription Factors Drives Transcriptional Outputs and Cytokine Specificity. *Immunity* **42**, 877-889 (2015).
116. J. Liu, X. Guan, X. Ma, Regulation of IL-27 p28 gene expression in macrophages through MyD88- and interferon-gamma-mediated pathways. *J Exp Med* **204**, 141-152 (2007).
117. O. Devergne *et al.*, Role of the TRAF binding site and NF-kappaB activation in Epstein-Barr virus latent membrane protein 1-induced cell gene expression. *J Virol* **72**, 7900-7908 (1998).
118. S. Wirtz *et al.*, EBV-induced gene 3 transcription is induced by TLR signaling in primary dendritic cells via NF-kappa B activation. *J Immunol* **174**, 2814-2824 (2005).
119. Q. Wang *et al.*, IL-27 signalling promotes adipocyte thermogenesis and energy expenditure. *Nature* **600**, 314-318 (2021).
120. Q. Chen *et al.*, Development of Th1-type immune responses requires the type I cytokine receptor TCCR. *Nature* **407**, 916-920 (2000).
121. A. Villarino *et al.*, The IL-27R (WSX-1) is required to suppress T cell hyperactivity during infection. *Immunity* **19**, 645-655 (2003).

122. H. Yoshida *et al.*, WSX-1 is required for the initiation of Th1 responses and resistance to *L. major* infection. *Immunity* **15**, 569-578 (2001).
123. M. Batten *et al.*, Interleukin 27 limits autoimmune encephalomyelitis by suppressing the development of interleukin 17-producing T cells. *Nat Immunol* **7**, 929-936 (2006).
124. J. S. Stumhofer *et al.*, Interleukin 27 negatively regulates the development of interleukin 17-producing T helper cells during chronic inflammation of the central nervous system. *Nat Immunol* **7**, 937-945 (2006).
125. Y. Cao, P. D. Doodles, T. T. Glant, A. Finnegan, IL-27 induces a Th1 immune response and susceptibility to experimental arthritis. *J Immunol* **180**, 922-930 (2008).
126. J. S. Stumhofer, C. A. Hunter, Advances in understanding the anti-inflammatory properties of IL-27. *Immunol Lett* **117**, 123-130 (2008).
127. S. Hamano *et al.*, WSX-1 is required for resistance to *Trypanosoma cruzi* infection by regulation of proinflammatory cytokine production. *Immunity* **19**, 657-667 (2003).
128. C. Hölscher *et al.*, The IL-27 receptor chain WSX-1 differentially regulates antibacterial immunity and survival during experimental tuberculosis. *J Immunol* **174**, 3534-3544 (2005).
129. E. S. Hwang, J. H. Hong, L. H. Glimcher, IL-2 production in developing Th1 cells is regulated by heterodimerization of RelA and T-bet and requires T-bet serine residue 508. *J Exp Med* **202**, 1289-1300 (2005).
130. A. V. Villarino *et al.*, IL-27 limits IL-2 production during Th1 differentiation. *J Immunol* **176**, 237-247 (2006).
131. T. Owaki *et al.*, IL-27 suppresses CD28-mediated [correction of medicated] IL-2 production through suppressor of cytokine signaling 3. *J Immunol* **176**, 2773-2780 (2006).
132. C. F. Anderson, J. S. Stumhofer, C. A. Hunter, D. Sacks, IL-27 regulates IL-10 and IL-17 from CD4⁺ cells in nonhealing *Leishmania major* infection. *J Immunol* **183**, 4619-4627 (2009).
133. A. P. Freitas do Rosário *et al.*, IL-27 promotes IL-10 production by effector Th1 CD4⁺ T cells: a critical mechanism for protection from severe immunopathology during malaria infection. *J Immunol* **188**, 1178-1190 (2012).
134. C. A. Hunter, R. Kastelein, Interleukin-27: balancing protective and pathological immunity. *Immunity* **37**, 960-969 (2012).
135. F. D. Liu *et al.*, Timed action of IL-27 protects from immunopathology while preserving defense in influenza. *PLoS Pathog* **10**, e1004110 (2014).
136. M. T. de Aquino *et al.*, IL-27 limits central nervous system viral clearance by promoting IL-10 and enhances demyelination. *J Immunol* **193**, 285-294 (2014).
137. C. Zhu *et al.*, An IL-27/NFIL3 signalling axis drives Tim-3 and IL-10 expression and T-cell dysfunction. *Nat Commun* **6**, 6072 (2015).
138. N. Chihara *et al.*, Induction and transcriptional regulation of the co-inhibitory gene module in T cells. *Nature* **558**, 454-459 (2018).
139. R. Salcedo *et al.*, Immunologic and therapeutic synergy of IL-27 and IL-2: enhancement of T cell sensitization, tumor-specific CTL reactivity and complete regression of disseminated neuroblastoma metastases in the liver and bone marrow. *J Immunol* **182**, 4328-4338 (2009).

140. Z. Liu *et al.*, IL-27 enhances the survival of tumor antigen-specific CD8⁺ T cells and programs them into IL-10-producing, memory precursor-like effector cells. *Eur J Immunol* **43**, 468-479 (2013).
141. Z. Liu, J. Yu, W. E. Carson, 3rd, X. F. Bai, The role of IL-27 in the induction of anti-tumor cytotoxic T lymphocyte response. *Am J Transl Res* **5**, 470-480 (2013).
142. M. Batten *et al.*, IL-27 supports germinal center function by enhancing IL-21 production and the function of T follicular helper cells. *J Exp Med* **207**, 2895-2906 (2010).
143. F. McNab, K. Mayer-Barber, A. Sher, A. Wack, A. O'Garra, Type I interferons in infectious disease. *Nat Rev Immunol* **15**, 87-103 (2015).
144. M. Colonna, G. Trinchieri, Y. J. Liu, Plasmacytoid dendritic cells in immunity. *Nat Immunol* **5**, 1219-1226 (2004).
145. L. B. Ivashkiv, L. T. Donlin, Regulation of type I interferon responses. *Nat Rev Immunol* **14**, 36-49 (2014).
146. N. Yan, Z. J. Chen, Intrinsic antiviral immunity. *Nat Immunol* **13**, 214-222 (2012).
147. J. W. Schoggins *et al.*, A diverse range of gene products are effectors of the type I interferon antiviral response. *Nature* **472**, 481-485 (2011).
148. M. D. Hyreza *et al.*, Distinct transcriptional profiles in ex vivo CD4⁺ and CD8⁺ T cells are established early in human immunodeficiency virus type 1 infection and are characterized by a chronic interferon response as well as extensive transcriptional changes in CD8⁺ T cells. *J Virol* **81**, 3477-3486 (2007).
149. M. Rotger *et al.*, Comparative transcriptomics of extreme phenotypes of human HIV-1 infection and SIV infection in sooty mangabey and rhesus macaque. *J Clin Invest* **121**, 2391-2400 (2011).
150. B. Jacquelin *et al.*, Nonpathogenic SIV infection of African green monkeys induces a strong but rapidly controlled type I IFN response. *J Clin Invest* **119**, 3544-3555 (2009).
151. J. R. Teijaro *et al.*, Persistent LCMV infection is controlled by blockade of type I interferon signaling. *Science* **340**, 207-211 (2013).
152. E. B. Wilson *et al.*, Blockade of chronic type I interferon signaling to control persistent LCMV infection. *Science* **340**, 202-207 (2013).
153. C. T. Ng *et al.*, Blockade of interferon Beta, but not interferon alpha, signaling controls persistent viral infection. *Cell Host Microbe* **17**, 653-661 (2015).
154. T. Wu *et al.*, The TCF1-Bcl6 axis counteracts type I interferon to repress exhaustion and maintain T cell stemness. *Sci Immunol* **1** (2016).
155. B. Fallet *et al.*, Interferon-driven deletion of antiviral B cells at the onset of chronic infection. *Sci Immunol* **1** (2016).
156. E. A. Moseman, T. Wu, J. C. de la Torre, P. L. Schwartzberg, D. B. McGavern, Type I interferon suppresses virus-specific B cell responses by modulating CD8(+) T cell differentiation. *Sci Immunol* **1** (2016).
157. S. Sammicheli *et al.*, Inflammatory monocytes hinder antiviral B cell responses. *Sci Immunol* **1** (2016).